

Root hydraulic conductivity and xylem sap levels of zeatin riboside and abscisic acid in ectomycorrhizal Douglas fir seedlings

BY MARK D. COLEMAN*, CAROLINE S. BLEDSOE
AND BARBARA A. SMIT

College of Forest Resources, University of Washington, Seattle, WA 98195, USA

(Received 26 July 1989; accepted 31 January 1990)

SUMMARY

Mechanistic hypotheses to explain mycorrhizal enhancement of root hydraulic conductivity (L_p) suggest that phosphorus (P) nutrition, plant growth substances and/or altered morphology may be responsible. Such ideas are based on work with VA (vesicular-arbuscular) mycorrhizas. Since VA mycorrhizas and ectomycorrhizas differ in many respects, they may alter host plant water uptake via different mechanisms. This paper examines L_p in various ectomycorrhizal associations while considering factors which are important to the VA mycorrhizal effect on L_p . Douglas fir *Pseudotsuga menziesii* (Mirb.) Franco seedlings inoculated with the ectomycorrhizal fungi *Laccaria bicolor* (Maire) Orton and *Hebeloma crustuliniforme* (Bull. ex St. Amans) Quel. and non-inoculated seedlings infected naturally with *Thelephora* were grown under three low levels of P fertilization (1, 10 and 100 μM P). Seedling morphology, tissue P levels, L_p and plant growth substance levels in xylem sap were measured after nine months growth. Increased tissue P and decreased root/shoot ratio correlated with increased L_p in each of the mycorrhizal treatments. When adjusted for the effect of these two factors, L_p of *Laccaria* and *Hebeloma* seedlings was still lower than the *Thelephora* seedlings. In a subsequent experiment, the L_p of seedlings with *Hebeloma* and *Rhizopogon vinicolor* Smith mycorrhizas was compared to the L_p of non-mycorrhizal seedlings (grown at 100 μM P) and no differences were found among treatments. The lack of an ectomycorrhizal effect on L_p is quite different from the enhancement of host L_p by VA mycorrhizas. Zeatin riboside concentrations of *Thelephora*- and *Hebeloma*-infected seedlings were similar, yet higher than with *Laccaria*. There was no relationship between plant growth substances and L_p in ectomycorrhizal Douglas fir, despite lower zeatin riboside concentrations for *Laccaria*-inoculated plants.

Key words: Abscisic acid, zeatin riboside, diurnal root conductance, ectomycorrhizas, phosphorus, root hydraulic conductivity.

INTRODUCTION

The anatomical position of mycorrhizas at the soil–root interface, with extensive hyphal connections emanating into the soil, suggests a role for these symbionts in plant water relations. Much of our understanding of mycorrhizal water relations comes from work with VA (vesicular-arbuscular) mycorrhizal associations. Water use is greater for VA mycorrhizal plants than for non-mycorrhizal plants. This result is an indirect effect of enhanced P status of VA mycorrhizal plants (Nelsen & Safir, 1982; Graham & Syvertsen, 1984; Koide, 1985; Fitter,

1988). Altered morphology, specifically root/shoot ratio, is also considered to be responsible for the VA mycorrhizal effect on plant water flux (Andersen *et al.*, 1988) and may also be related to enhanced P status. In addition, VA mycorrhizal effects on water flux have been attributed to an altered balance of plant growth substances (Levy & Krikun, 1980; Allen *et al.*, 1981). The latter hypothesis has, to our knowledge, not been rigorously tested by concurrent measurement of water flux and plant growth substance levels.

The anatomy of VA mycorrhizas differs from that of ectomycorrhizas. The VA habit is characterized by intercellular and interprotoplasmic colonization of the cortex of host feeder roots; external hyphae emanate into the soil. Ectomycorrhizal roots also maintain soil hyphae, but a large amount of the

* Present address: United States Department of Agriculture, Forestry Sciences Laboratory, P.O. Box 898, Rhinelander, WI 54501.

fungus material ensheathes the root in a hyphal envelope with variable intercellular penetration of the outer cortex. Such a difference in structure may have correspondingly divergent effects on function.

Ectomycorrhizal associations can have a positive, negative or neutral effect on plant water use relative to non-infected host plants (Sands & Theodorou, 1978; Dixon *et al.*, 1980; Sands, Fiscus & Reid, 1982; Parke, Lindermann & Black, 1983; Coleman, 1988). Such differences among reports may result from variable effects of fungal species on host metabolism. In order to determine whether ectomycorrhizal associations behave as VA mycorrhizal associations do, we tested the hypothesis that root hydraulic conductivity (L_p) of ectomycorrhizal root systems infected with various fungi is greater than that of non-mycorrhizal root systems. Ectomycorrhizas are known to affect seedling size and tissue P content similarly to VA mycorrhizal associations, especially under low P levels (Harley & Smith, 1983). Therefore, hydraulic properties of roots with different ectomycorrhizal symbionts were measured while accounting for the effects of seedling size and P content. Phosphorus was applied at low levels to test the efficiency of different mycorrhizal associations to enhance P availability in the range of soil solution levels. Finally, plant growth substance levels were determined in xylem sap expressed from roots during L_p experiments, allowing concurrent measurements of growth substances moving in the transpiration stream and water flux.

MATERIALS AND METHODS

Fungal material

Two ectomycorrhizal fungi, *Hebeloma crustuliniforme* (Bull. ex St Amans) Quel. [S166, ATCC* no. 46332, isolated from a sporocarp collected under Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) near Woods Creek Road, Benton County, OR, USA at an elevation of 490 m] and *Laccaria bicolor* (Maire) Orton [MC102486-1, ATCC no. 64925, isolated from a sporocarp collected under Douglas fir and western hemlock (*Tsuga mertensiana* [Bong.] Carr.) near upper Devil's Club Creek, Chelan County, WA, USA at an elevation of 1250 m], were used as inoculum for seedling material grown in the greenhouse. Mycelial inoculum was grown on a vermiculite support base (Marx & Bryan, 1975). Canning jars (4 l) containing 1400 cm³ coarse vermiculite, 100 cm³ sieved Canadian peat moss and 880 ml liquid modified Melin-Norkrans media were capped with ventilated lids and autoclaved. Each ventilated canning jar lid contained a 16 mm diameter piece of glass tubing sealed into a hole with a culture tube cap covering the exposed end. *Rhizo-*

pogon vinicolor Smith spores collected under Douglas fir in Polk County, OR, USA, and mycelial inoculum of the above *Hebeloma* isolate were used to inoculate seedlings grown in the growth chamber.

Plant material

Stratified Douglas fir seed from a half-sib source located in the Randal Ranger District, Gifford Pinchot National Forest, WA, USA at an elevation of 610 m was germinated in 65 ml Leach tubes (Ray Leach Nursery, Canby, OR, USA) containing sterilized vermiculite:peat moss (1:1) potting soil. Following germination, seedlings were watered three times a week, one being a fertilization with complete nutrient solution. The nutrient solution contained 3.57 mM NH₄NO₃, 1.61 mM KH₂PO₄, 166 µM CaSO₄·2H₂O, 40 µM MgSO₄·7H₂O, 1 µM H₃BO₃, 0.08 µM MnSO₄·H₂O, 0.08 µM ZnSO₄, 0.04 µM CuSO₄, 0.04 µM MoO₃ and 0.18 mg l⁻¹ Sequestrene (330-Fe), Ciba-Geigy). Seven weeks after sowing, seedlings were transplanted into 550 ml Deepot tubes (McConkey & Co., Inc., Sumner, WA, USA) containing one part leached vermiculite-based inoculum mixed with nine parts sterilized potting soil. Seedlings were either inoculated with *Laccaria* or *Hebeloma* or with non-viable inoculum (containing a fungus which did not colonize roots). Following transplanting and inoculation, the level of nitrogen (N) applied to all mycorrhizal treatments was reduced to 1.79 mM NH₄NO₃ in the nutrient solution to encourage mycorrhizal formation.

In a greenhouse experiment the effect of low levels of P nutrition and ectomycorrhizal infection was examined in a two-way factorial design which crossed three mycorrhizal treatments (*Laccaria*, *Hebeloma* and non-inoculated) with three levels of P (1, 10 and 100 µM P). The P was applied as KH₂PO₄ with variable amounts of KCl added to maintain potassium concentration at 0.77 mM. The greenhouse photoperiod was extended to 15 h using high-pressure sodium vapour lamps providing photosynthetic photon flux density of 700 µmol m⁻² s⁻¹. Measurements were taken on seedlings in this complete factorial design after nine months.

Non-inoculated seedlings became contaminated in the greenhouse growth environment; identical techniques were therefore used to grow seedlings in a growth chamber where contamination with airborne spores was avoided. *Hebeloma* and *Rhizopogon* (the latter applied as a spore suspension) were compared to non-inoculated seedlings. Both the *Rhizopogon* and non-inoculated seedlings received autoclaved inoculum in the transplant media. Seedlings were raised at only one level of P (100 µM P). The growth chamber (EGC, Chagrin Falls, OH, USA) maintained respective day/night temperature at 23/18 °C and relative humidity at 40/60 %. The photoperiod was 15 h at 600 µmol m⁻² s⁻¹ photosynthetic photon

* Isolates are deposited in the American Type Culture Collection, Rockville, MD, USA.

flux density using mixed sodium vapour and metal halide lamps.

Morphological measurements

Immediately prior to root hydraulic conductivity measurements, seedling height and root collar diameter were measured. Following measurement of L_p on severed root systems, leaf and stem dry weight were measured and the total root system was separated into coarse and fine (< 1 mm diameter) roots. Fine roots were separated and spread on a tray (30 × 40 cm) containing a 10 × 10 grid. Two subsamples were obtained by selecting roots from randomly chosen grid squares until at least 10% of the root system fresh weight was obtained. Root length was measured on one subsample using a digitizing pad (Videoplan, Carl Zeiss, Inc., Thornwood, NY, USA) to trace root images made by an office photocopier. Length and dry weight of the subsample provided an estimate of specific root length. Mycorrhizal infection – the percentage of root tips that were mycorrhizal – was counted on the other subsample. Dry weights were obtained for foliage, stem and fine and woody roots. Total fine root length was calculated as the product of fine root weight and specific root length. Leaf area was determined from foliage dry weights using a specific leaf area of 71.17 cm² g⁻¹ (Coleman, 1988). Leaf and fine root tissue were analysed for N and P.

Root hydraulic conductivity

Root hydraulic conductivity was measured using the pressure chamber technique of Fiscus (1977). To avoid root injury and disruption of the mycorrhizal hyphal system, the root plugs containing soil and intact undisturbed root system were gently removed from containers and wrapped in cheese cloth. Under the high soil moisture conditions of these experiments (> -0.01 MPa soil water potential), the hydraulic conductivity of the peat-vermiculite potting soil is greater than 27 times that of the root system (Farnum, 1977) and thus, could be ignored. Six separate detached seedling root systems were sealed into the lid of a 20 l pressure chamber (Smit & Stachowiak, 1988) with the cut stems protruding through six fittings in the top. Roots were submerged in aerated, temperature controlled (20 ± 0.1 °C) nutrient solution with pH adjusted to 5.5 ± 0.05 with KOH. The solution contained the same salt concentrations as the post-inoculation fertilizer solution except KH₂PO₄ was excluded and 0.77 mM KCl was provided. Root systems were prepared the night before an experiment and placed under 0.4 MPa pressure overnight (10.5 h) to assure a stable reading and to collect xylem sap for plant growth substance analysis. Flow was measured gravimetrically at 0.4, 0.5 and 0.6 MPa pressures after at least 2 h equi-

libration providing a linear ($r^2 > 0.92$) pressure-flow relationship (Fiscus, 1977). Four flow-rate determinations of 10 min each were made at each pressure. Root hydraulic conductance (L , mg s⁻¹ MPa⁻¹) was calculated from the slope of mean flow rate as a function of applied pressure for the entire root system. This conductance value was then used to calculate L_p (mg s⁻¹ m⁻¹ MPa⁻¹) by division with fine root length.

Plant growth substance assays

The levels of abscisic acid (ABA) and zeatin riboside (ZR) were measured in xylem sap collected from detached roots of 9-month-old seedlings prior to measurement of root hydraulic conductivity. The first sap to be extruded following cutting of the stem was collected into methanol on ice while root systems were equilibrating at 0.4 MPa applied pressure; 1–7 ml was obtained from each root system. Samples were lyophilized and stored at -20 °C until analysed. The sap was resuspended in PBS buffer (0.01 M Na₂PO₄, 0.15 M NaCl, pH 7.4) to the desired concentration for analysis. A competitive binding radioimmunoassay was used for estimating ABA concentrations while the ZR concentration in the sap was measured by an enzyme-linked immunosorbent assay (Smit, Neuman & Stachowiak, 1990). Recovery of a standard spike at various sap concentrations and parallelism of standard curves in sap or PBS demonstrated no interference of the Douglas-fir sap with the immunological tests, making assays of crude samples possible. The xylem sap was assayed at ×1 concentration for ABA and at ×2 for ZR.

Statistical analysis

Data from the greenhouse experiment were statistically analyzed using a completely random, two-way factorial design (Steel & Torrie, 1980) with P fertilizer (three levels) and mycorrhizal inoculation (three levels) as the two treatment factors. The raw data from 7 seedlings in each treatment were used in the analysis for all variables except nutrient content and plant growth substance levels where only 5 of the 7 seedlings were chemically analysed. Data from the growth chamber experiment were examined in a one-way analysis of variance with five replicates per treatment.

Covariate analysis (Steel & Torrie, 1980; Kleinbaum & Kupper, 1978) allowed comparisons of L_p among mycorrhizal treatments (the dependent variable) while adjusting for correlations between L_p and either P concentration or root/shoot ratio. The latter variables are said to covary with the dependent variable. The analysis assumes that the linear relationship between the dependent variable and the covariate is the same, except for the vertical position (i.e. the y-intercept), for each treatment. In other

words the analysis assumes that regressions have the same slopes among treatments. The result of the covariate analysis determines if the vertical position of the regression line differs among treatments. The assumption of parallel slopes was confirmed for our data by testing regression lines for parallelism (Kleinbaum & Kupper, 1978).

Mean comparisons for the factorial and one-way analysis of variance were performed using Student-Neuman-Keuls test (Steel & Torrie, 1980). For the covariate analysis, mycorrhizal treatments were compared using two orthogonal comparisons to test the difference between (1) inoculated and non-inoculated treatments, and (2) the *Laccaria* and the *Hebeloma* treatments. All analyses were calculated using SYSTAT statistical package (Systat, Inc., Evanston, IL, USA).

RESULTS

Seedling morphology and nutrition

In the greenhouse experiment, inoculation with *Hebeloma crustuliniforme* and *Laccaria bicolor* was very successful (Table 1); infection levels were 85–87%. Nevertheless, roots in the non-inoculated treatment were partially mycorrhizal (40%) with aerially dispersed fungi. Fruit bodies of *Thelephora*

and *Inocybe* were observed in these containers. The dominating number of fruit bodies were *Thelephora* so this treatment will be referred to with that species name. *Hebeloma* seedlings had significantly smaller shoot weights, but other morphological variables were statistically similar. Besides the qualitative differences in root tip morphology among individual root symbionts, there were no quantitative root architectural differences. Variables such as fine root length (Table 1) and specific root length were not different among mycorrhizal treatments. Specific root length averaged $25 \pm 1 \text{ m g}^{-1}$ for each treatment combination and was very similar to previous measurements on Douglas fir (Coleman, 1988). Phosphorous treatment levels had no measurable effect on mycorrhizal formation. The $100 \mu\text{M P}$ seedlings were larger than seedlings in the lower P levels (Table 1).

For seedlings grown in the growth chamber, infection was high in the inoculated treatments while the non-inoculated seedlings were free of airborne mycorrhizal contamination (Table 2). Thus the non-inoculated seedlings grown in the growth chamber were truly non-mycorrhizal. *Hebeloma*- and *Rhizopogon*-inoculated seedlings had smaller root systems and lower root/shoot ratios than the non-mycorrhizal seedlings (Table 2).

Table 1. Morphological data for 9-month-old seedlings grown in the greenhouse and treated with various P levels. Mycorrhizal inoculation with *Laccaria bicolor* and *Hebeloma crustuliniforme* was compared to seedlings inoculated naturally with *Thelephora*.

	Mycorrhizal infection (%)	Dry weight (g)			Fine root length (m seedling ⁻¹)	Root/shoot ratio
		Root	Shoot	Total		
Applied P level (μM)						
1	70a	2.39ab	2.66b	5.05b	39.5a	0.91a
10	70a	2.08b	2.66b	4.74b	31.6b	0.79b
100	72a	2.69a	3.50a	6.19a	43.2a	0.77b
Mycorrhizal fungus						
<i>Laccaria</i>	85a	2.47a	2.95ab	5.41a	38.9a	0.84a
<i>Hebeloma</i>	87a	2.26a	2.72b	4.98a	36.2a	0.85a
<i>Thelephora</i>	40b	2.44a	3.15a	5.59a	39.2a	0.77a

Since there were no significant interactions between treatments, the inoculation treatments within P treatments and P treatments within inoculation treatments were pooled.

Values in the same column followed by the same letter are not significantly different ($P = 0.05$, Newman-Keuls mean comparison; $n = 7$).

Table 2. Morphological data for 9-month-old seedlings raised in the growth chamber. Mycorrhizal inoculations with *Hebeloma crustuliniforme* and *Rhizopogon vinicolor* were compared to non-mycorrhizal seedlings

	Mycorrhizal infection (%)	Dry weight (g)			Fine root length (m seedling ⁻¹)	Root/shoot ratio
		Root	Shoot	Total		
<i>Hebeloma</i>	96a	2.35b	2.32b	4.67b	40.6a	1.02b
<i>Rhizopogon</i>	85b	2.29b	2.86a	5.16b	30.6a	0.81b
Non-mycorrhizal	0c	3.67a	2.66a	6.34a	52.0a	1.38a

Values in the same column followed by the same letter are not significantly different ($P = 0.05$, Newman-Keuls mean comparison; $n = 5$).

Table 3. Nutritional data, on a dry-weight basis, for 9-month-old seedlings grown in the greenhouse (see Table 1 for an explanation of treatments and interactions)

	Nutrient concentration (%)		Nutrient content (mg seedling ⁻¹)	
	Root	Leaf	Root	Leaf
Phosphorus				
Applied P level (μM)				
1	0.069 b	0.048 b	1.15 b	0.834 b
10	0.071 b	0.052 b	1.05 b	0.993 b
100	0.087 a	0.067 a	1.67 a	1.73 a
Mycorrhizal fungus				
<i>Laccaria</i>	0.070 b	0.048 b	1.27 a	1.03 a
<i>Hebeloma</i>	0.076 ab	0.061 a	1.26 a	1.26 a
<i>Thelephora</i>	0.081 a	0.058 a	1.33 a	1.25 a
Nitrogen				
Applied P level (μM)				
1	0.88 a	1.23 a	14.8 b	21.8 a
10	0.95 a	1.27 a	14.0 b	24.4 a
100	0.95 a	0.817 b	18.5 a	20.6 a
Mycorrhizal fungus				
<i>Laccaria</i>	0.99 a	0.97 a	17.8 a	20.2 a
<i>Hebeloma</i>	0.92 a	1.19 a	15.1 a	23.5 a
<i>Thelephora</i>	0.88 a	1.17 a	14.4 a	23.0 a

Values in the same column followed by the same letter are not significantly different ($P = 0.05$, Newman-Keuls mean comparison; $n = 5$).

Average foliar P concentrations for greenhouse grown seedlings in the 100 μM P treatment were greater than those of lower applied P levels (Table 3). *Thelephora* seedlings had statistically similar P concentrations to *Hebeloma*-inoculated seedlings but greater than *Laccaria*-inoculated seedlings. Nitrogen concentrations were similar among all treatment factors, aside from the dilution which occurred in the larger seedlings of the 100 μM P treatment. The total uptake of N and P was equal among mycorrhizal treatments.

Tissue P concentration was significantly correlated

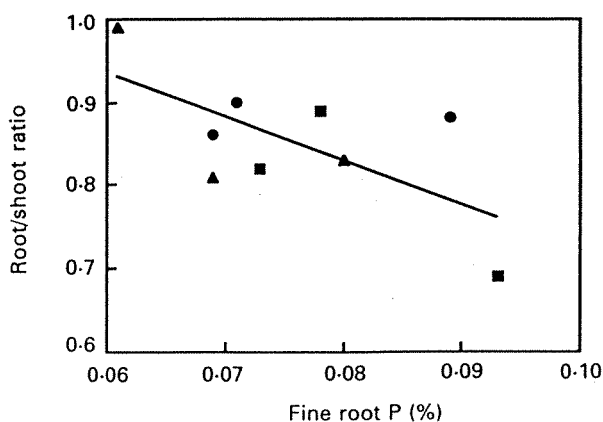


Figure 1. Root/shoot ratio as a function of fine root P concentration. Each point is the mean of $n = 5$ measurements. The line represents the regression ($r^2 = 0.432$) through these mean values. \blacktriangle , *Laccaria*; \bullet , *Hebeloma*; \blacksquare , *Thelephora*.

with morphological variables measured. Particularly relevant to the L_p results is the relationship between fine root P concentration and root/shoot ratio. The relationship for raw data was statistically significant ($P = 0.017$), but the variability was large ($r^2 = 0.125$). Mean values are presented in Figure 1.

Hydraulic conductivity

The diurnal pattern of xylem sap flux from detached seedling root systems exhibited a morning maximum and an afternoon minimum. This pattern was not altered by initiating experiments at different times of day nor by constant light or temperature treatments during the week prior to measurements (Fig. 2). To control for this daily fluctuation in flow, measurements for each pressure were made at the same time each (0.4 MPa, 08.00 h; 0.5 MPa, 11.00 h; 0.6 MPa, 14.00 h).

In greenhouse-grown seedling root systems, L was significantly related to both fine root length ($P < 0.001$, $r^2 = 0.185$) and leaf area ($P < 0.001$, $r^2 = 0.215$). The low coefficient of determination (r^2) values shows the large variability present in the data. In spite of this variability it is important to account for plant size when comparing treatments. Root conductance was normalized by dividing L by root length and was designated L_p . Similar patterns were found when L was normalized by leaf area (data not shown).

The factorial analysis of variance for L_p indicated

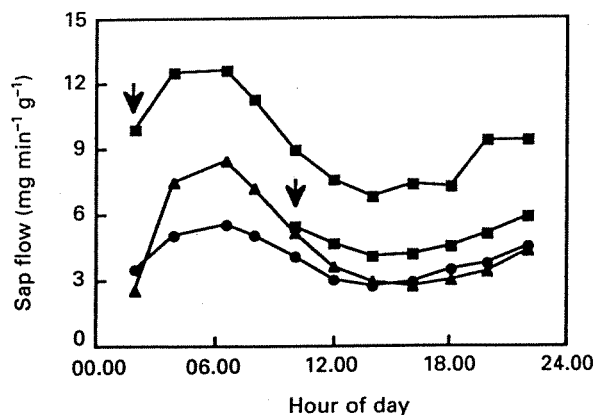


Figure 2. Diurnal variation in flux of sap from pressurized (0.5 MPa) Douglas-fir seedling root systems. Measurements of sap flow commenced at either 01.00 or 10.00 h as indicated by arrows. Pretreatments lasted for 1 week before measurement where light and temperature either followed daily patterns or were constant. There were 3 pretreatments: variable light and temperature (—■—), only variable light (—▲—), or only variable temperature (—●—). Photoperiod started at 05.00 h and continued until 21.00 h.

that both the mycorrhizal treatment effect (F ratio = 6.71, $P = 0.002$) and the P treatment effect (F ratio = 4.18, $P = 0.020$) were significant. There was no interaction between treatments (F ratio = 0.50, $P = 0.736$), and therefore, only the mean values for the main effects are presented (Table 4). Lower L_P values were found for the *Laccaria* than the *Thelephora* seedlings and increased applied P resulted in increased L_P .

To examine the mechanism of the mycorrhizal effect on L_P , morphological and nutritional variables were examined. There was an inverse relationship between L_P and root/shoot ratio (Fig. 3). A covariate analysis was used on the raw data to determine if greater root/shoot ratios in *Laccaria* and *Hebeloma*

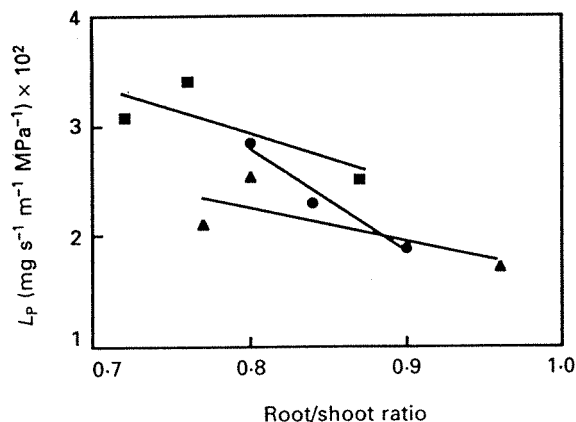


Figure 3. Root hydraulic conductivity (L_P) as a function of root/shoot ratio. Points are the mean of $n = 7$ replicates. Lines are regressions through these mean values for each mycorrhizal treatment [*Laccaria*, (▲), $r^2 = 0.580$; *Hebeloma*, (●), $r^2 = 0.962$; *Thelephora* (■), $r^2 = 0.654$; overall (line not shown), $r^2 = 0.607$].

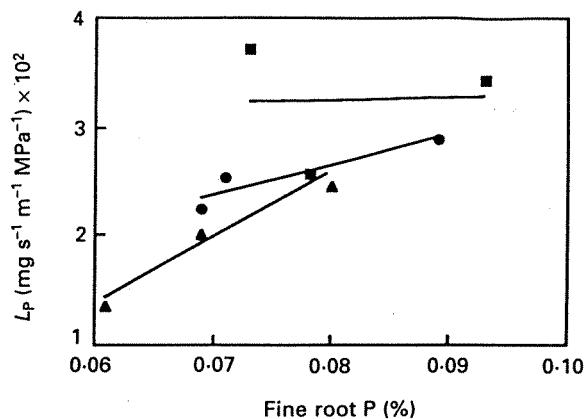


Figure 4. Root hydraulic conductivity (L_P) as a function of P concentration of fine roots. Points are the mean of $n = 5$ replicates. Lines are regressions through these mean values for each mycorrhizal treatment [*Laccaria* (▲), $r^2 = 0.959$; *Hebeloma* (●), $r^2 = 0.870$; *Thelephora* (■), $r^2 = 0.003$; overall (line not shown), $r^2 = 0.465$].

treatments were responsible for their lower L_P values. The assumption of parallel slopes among mycorrhizal treatments was accepted. The covariate analysis showed that mean L_P values were statistically equal for *Laccaria* and *Hebeloma* treatments adjusted for a common (mean) root/shoot ratio (0.0213 and 0.0237 $\text{mg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$ respectively), yet significantly lower than the adjusted mean for *Thelephora*-infected seedlings (0.0293 $\text{mg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$).

The pattern of increased conductance with increased P application was apparent on a whole root system (L) as well as a root length (L_P) basis (Table 4). Since tissue P concentration was positively correlated with L_P (Fig. 4) and quite variable within each mycorrhizal treatment, another covariate analysis was used on the raw data. The assumption of parallel slopes was accepted based on test results. Mean L_P values indicated that the inoculated treatments (*Laccaria*, 0.0201; *Hebeloma*, 0.0254 $\text{mg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$) were statistically equal, yet still significantly lower than *Thelephora* seedlings (0.0315 $\text{mg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$) after being adjusted by the covariate analysis for mean fine root P concentration. Therefore, when the effects of root/shoot ratio and P concentration were accounted for, the *Thelephora* seedlings, which had lower levels of mycorrhizal infection, had significantly higher L_P .

Seedlings raised in the growth chamber and inoculated with *Rhizopogon* had lower mean L values than non-mycorrhizal seedlings (Table 5), but L_P values were not different among treatments. There was no relationship between root/shoot ratio and L_P in these seedlings.

Plant-growth substances

Fertilizer treatment had no significant effect on either ZR or ABA levels in the xylem sap so data

Table 4. Hydraulic conductance of whole seedlings root systems (L) and root hydraulic conductivity expressed on a unit fine root length basis (L_p) for greenhouse-grown material (see Table 1 for an explanation of treatments and interactions)

	L ($\text{mg s}^{-1} \text{MPa}^{-1}$)	L_p ($\text{mg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$) $\times 10^2$
Applied P level (μM)		
1	0.775b	2.04b
10	0.811b	2.59ab
100	1.14a	2.81a
Mycorrhizal fungus		
<i>Laccaria</i>	0.778b	2.11b
<i>Hebeloma</i>	0.834b	2.34ab
<i>Thelephora</i>	1.11a	2.99a

Values in the same column followed by the same letter are not significantly different ($P = 0.05$, Newman-Keuls mean comparison; $n = 7$).

from the P treatments were pooled within each mycorrhizal treatment. Xylem sap concentrations of ZR for *Laccaria*-inoculated seedlings were less than half of the other treatments (Fig. 5). Since it is not known whether concentration or transport rate of growth substances in the xylem is more important to function, xylem sap concentrations were divided by

fine root weight (an estimate of the amount produced per unit root) or multiplied by sap flow rate (an estimate of the amount delivered to the shoot per unit time). The adjusted ZR values were lower in *Laccaria*- than in *Hebeloma*-inoculated seedlings. There was no effect of mycorrhizal treatment on ABA concentration or on adjusted ABA levels (Fig. 5).

DISCUSSION

Diurnal pattern of xylem sap flux

The diurnal patterns of xylem sap flux observed in preliminary experiments are in contrast to previous reports where maximum conductivity was observed in midday (Parsons & Kramer, 1974; Fiscus, 1986). A midday maximum for root conductance coincides with maximum transpirational demand. The Douglas fir pattern displayed maximum flux at the end of the dark period when transpirational demand was low (Fig. 2). Early morning maximum root conductance may enhance the recharge of water storage compartments during the dark period. It is doubtful that this water flux pattern is an artifact of the root excision and measurement technique since it was consistent regardless of the starting time.

Morphological control of L_p

Seedling morphology has been shown to affect L_p (Fiscus & Markhart, 1979; Andersen *et al.*, 1989). Root/shoot ratio is inversely related to L_p in Douglas fir (Fig. 3), as found by Anderson *et al.* (1989) for *Fraxinus pennsylvanica*. Such morphological effects may account for the VA mycorrhizal effect on L_p . VA mycorrhizal infection has resulted in decreased (Graham *et al.*, 1987; Levey *et al.*, 1983) and increased (Hardie & Leyton, 1981) L_p , but as Andersen *et al.* (1988) point out, accounting for

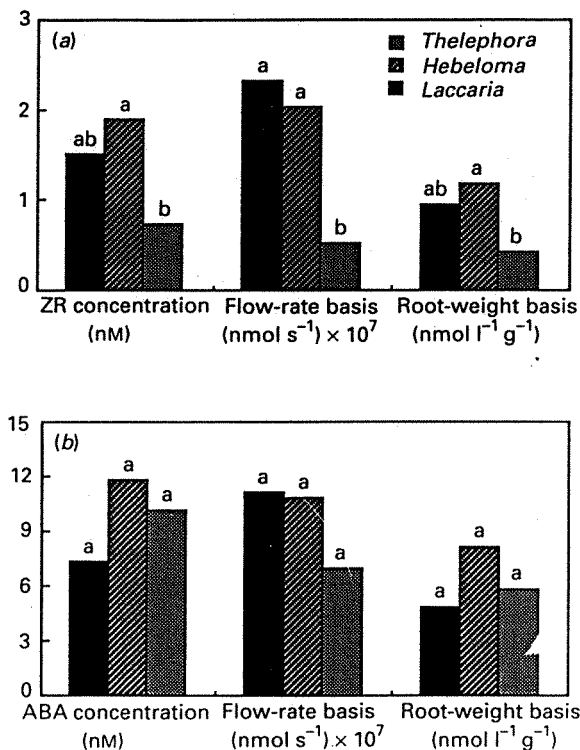


Figure 5. Concentration of zeatin riboside (a) and abscisic acid (b) in sap from pressurized roots. Concentrations were expressed in nM or normalized on a flow rate and on a root-weight basis. Columns in each group with the same letter are not significantly different ($P = 0.05$, Newman-Keuls mean comparison; $n = 15$). Significant differences for zeatin riboside concentration using $P = 0.05$ were non-significant, $P = 0.10$ was used. ■, *Thelephora*; ▨, *Hebeloma*; ▤, *Laccaria*.

differences in root/shoot ratio may equalize such differences in L_p between VA mycorrhizal and non-mycorrhizal plants. Nevertheless, the effect of the *Laccaria* and *Hebeloma* treatments in lowering L_p was not due entirely to the difference in root/shoot ratio since differences persisted when root-shoot effects were adjusted for with covariate analysis.

The growth chamber experiment compared non-mycorrhizal seedlings to largely mycorrhizal seedlings. L_p was not different among mycorrhizal treatments even though L was lower for infected seedlings, especially with *Rhizopogon* (Table 5). Based on this evidence it must be concluded that mycorrhizal infection had no detectable effect on L_p ; rather the major effect was to decrease root system size and root/shoot ratio relative to the non-mycorrhizal seedlings and thereby decrease L .

The response of the non-inoculated seedlings in the growth chamber (non-mycorrhizal) and in the greenhouse (40% infected with *Thelephora*) was quite different regarding root/shoot ratio and L_p . Comparisons of the non-inoculated treatments were made relative to the *Hebeloma* inoculation, since *Hebeloma* inoculation was used in both experiments. In the case of *Thelephora* seedlings, L_p appeared to be greater relative to *Hebeloma* (Table 4) and root/shoot ratio statistically equal (Table 1). For growth chamber grown non-mycorrhizal seedlings, L_p was statistically equal to *Hebeloma* (Table 5) and root/shoot ratio was greater (Table 2). Therefore, 40% infection with *Thelephora* affected seedling morphology and root hydraulic properties differently than did the other mycorrhizal infections. Root/shoot ratio and L_p were related (Fig. 3) for greenhouse, but not growth chamber grown seedlings, therefore, caution should be exercised in suggesting a mycorrhizal treatment effect on L_p based on root/shoot ratio differences in growth chamber raised seedlings. Variable results in ectomycorrhizal water relations of Douglas fir were previously reported to result from different fungal associates (Parke *et al.*, 1983). Such difference among associates may explain the conflicting results of other ectomycorrhizal water relations papers

(Sands & Theodorou, 1978; Dixon *et al.*, 1980; Sands *et al.*, 1982).

Phosphorus nutrition and L_p

Hydraulic conductivity through detached root systems increases with increased P levels in nutrient solutions (Radin & Eidenbock, 1984) or tissue (Andersen *et al.*, 1989). Increases in tissue P concentrations have been shown to account for a large amount of the VA mycorrhizal effect measured in L_p (Graham & Syvertsen, 1984) or whole plant water relations (Nelsen & Safir, 1982; Koide, 1985; Fitter, 1988), with some exceptions (Augé, Schekel & Wample, 1986).

Leaf P concentrations in this experiment were very low for Douglas fir (van den Driessche, 1984). The low P values and the limited response of tissue P levels to increased applied P was most likely due to the very low levels of P applied. The applied P levels were designed to provide a range that was representative of soil solution concentrations. Under low levels of P fertility mycorrhizal infection was expected to improve host P nutrition and accentuated any P-dependent differences in L_p among mycorrhizal treatments.

Even though leaf P levels were low, L_p did increase with both increased levels of P application (Table 4) and fine root P concentration (Fig. 4). Both of the inoculated treatments had mean L_p values which were positively correlated with mean fine root P (*Laccaria*, $r^2 = 0.959$; *Hebeloma*, $r^2 = 0.870$) but not for the *Thelephora* seedlings ($r^2 = 0.003$). Combining all treatment means results in a positive correlation between mean L_p and mean fine root P ($r^2 = 0.465$). This correlation is consistent with previous results on *Gossypium hirsutum* and *Fraxinus pennsylvanica* (Radin & Eidenbock, 1984; Andersen *et al.*, 1989).

A partial explanation for the lower L_p values of the *Laccaria* treatment, compared to the other treatments, is the lower fine root P level (Fig. 4, Table 3). However, covariate analysis demonstrated that fine root P concentration does not explain all of the

Table 5. Hydraulic conductance of whole seedlings root systems (L) and root hydraulic conductivity expressed on a unit fine root length basis (L_p) for mycorrhizal and non-mycorrhizal seedlings raised in a growth chamber

	L ($\text{mg s}^{-1} \text{MPa}^{-1}$)	L_p ($\text{mg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$) $\times 10^2$
<i>Hebeloma</i>	1.32 ab	3.50 a
<i>Rhizopogon</i>	0.886 b	2.98 a
Non-mycorrhizal	1.64 a	3.43 a

Values in the same column followed by the same letter are not significantly different ($P = 0.05$, Newman-Keuls mean comparison; $n = 5$).

depression of L_p for *Laccaria* and *Hebeloma* treatments. The two inoculated treatments had significantly lower L_p when adjusted for fine root P concentration. Since root/shoot ratios may be responsible for a portion of the difference of L_p among mycorrhizal treatments, root/shoot ratio was included with fine root P in a covariate analysis containing two independent variables. Such an analysis did not account for any more of the difference in L_p among mycorrhizal treatments than either of the models including fine root P or root/shoot ratio alone. This consequence may be attributed to the dependence of fine root P and root/shoot ratio on one another (Fig. 1). It was, therefore, concluded that although fine root P or root/shoot ratio could account for some of the difference between mycorrhizal treatments, there was a decline in L_p for *Laccaria* and *Hebeloma* relative to the *Thelephora* seedlings which was unexplained by either of these variables.

Plant-growth substances

Plant-growth substances were measured to test the hypothesis that mycorrhizal fungi alter levels of such compounds which, in turn, alter water flux through mycorrhizal plants (Levy & Krikun, 1980; Allen *et al.*, 1981). We measured ABA and ZR because of their effect on plant water relations and their reported movement from root to shoot in the xylem sap (Mansfield, 1987). ZR, which is synthesized in plant roots, is considered the major cytokinin moving in the xylem (Torrey, 1976), and so was selected for study. This does not imply that ZR is the active form of cytokinin or the only form produced by roots or mycorrhizal fungi. ABA is synthesized in the roots of several species and it has been hypothesized to be active in stomatal functioning (Davies *et al.*, 1987). In addition, L_p is affected by exogenous ABA levels (Markhart *et al.*, 1979; Fiscus, 1981).

In VA mycorrhizal plants, root and leaf tissue ABA levels were reduced (Allen, Moore & Christensen, 1982) and cytokinin levels were increased in root and leaf tissue (Allen, Moore & Christensen, 1980; Dixon, Garrett & Cox, 1988a) and in sap (Dixon, Garrett & Cox, 1988b). In our results neither P nor mycorrhizal treatments affected ABA levels in xylem sap. ZR levels were also unaffected by P treatments but were lower for *Laccaria*-inoculated seedlings than for the other two treatments (Fig. 5). These lower levels were not correlated with any other variable measured including root system size, P nutrition and L_p . It was not possible, however, to compare plant growth substance levels in mycorrhizal and non-mycorrhizal seedlings due to contamination of non-inoculated seedlings. Such contamination may have an unknown effect on host plant growth substance physiology.

Regardless of the contamination in the non-

mycorrhizal seedlings some conclusions can be drawn from these measurements of plant growth substances. There appears to be a difference in xylem sap concentrations of ZR for Douglas fir heavily infected with *Laccaria* compared to a heavy *Hebeloma* infection or a moderate *Thelephora* infection. The differences between these fungi further demonstrates that Douglas fir metabolism can be influenced by physiological diversity of fungal symbionts, similar to the effect on L_p noted above, and other aspects of host physiology (Bledsoe *et al.*, 1989). Equal ABA levels suggest that different ectomycorrhizal fungi either have the same influence or have no detectable influence on the balance of this growth substances in Douglas fir xylem sap.

CONCLUSIONS

Douglas-fir seedlings infected moderately with naturally occurring *Thelephora* had greater L_p than heavily infected *Hebeloma* or *Laccaria* seedlings.

L_p increased as P concentration increased and as root/shoot ratio decreased in Douglas fir seedlings, but such relationships could not account for differences in L_p among the different mycorrhizal fungi infecting root systems.

Infection with *Hebeloma* and *Rhizopogon* resulted in decreased root/shoot ratio and decreased L_p , but had no measureable effect on L_p relative to non-mycorrhizal seedlings.

Infection with *Laccaria* resulted in lower levels of zeatin riboside in the xylem sap of Douglas fir. The lower level of this growth substance was not related to the water-flux parameters measured.

ACKNOWLEDGEMENTS

The reviews of Drs Christen P. Andersen and Albert H. Markhart, III were very helpful in the preparation of this manuscript. Dr Dawn S. Neuman provided invaluable technical assistance in the evaluation of plant growth substances. Funding was provided by National Science Foundation grant no. BSR-8510858.

REFERENCES

- ALLEN, M. F., MOORE, T. S. & CHRISTENSEN, M. (1980). Phytohormone changes in *Bouteloua gracilis* infected by vesicular-arbuscular mycorrhizas. I. Cytokinin increases in the host plant. *Canadian Journal of Botany* **58**, 371–374.
- ALLEN, M. F., MOORE, T. S. & CHRISTENSEN, M. (1982). Phytohormone changes in *Bouteloua gracilis* infected vesicular-arbuscular mycorrhizas. II. Altered levels of gibberellin-like substances and abscisic acid in the host plant. *Canadian Journal of Botany* **60**, 468–471.
- ALLEN, M. F., SMITH, W. K., MOORE, T. S. & CHRISTENSEN, M. (1981). Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis* H.B.K. *Lag ex Steud. New Phytologist* **88**, 683–693.
- ANDERSEN, C. P., MARKHART, A. H., DIXON, R. K. & SUCCOFF, E. I. (1988). Root hydraulic conductivity of vesicular-arbuscular mycorrhizal green ash seedlings. *New Phytologist* **109**, 465–471.

- ANDERSEN, C. P., SUCCOFF, E. I., DIXON, R. K. & MARKHART, A. H. (1989). Effects of phosphorus deficiency on root hydraulic conductivity in *Fraxinus pennsylvanica*. *Canadian Journal of Botany* **67**, 472-476.
- AUGÉ, R. M., SCHEKEL, K. A. & WAMPLE, R. L. (1986). Greater leaf conductance of well-watered VA mycorrhizal rose plants is not related to phosphorus nutrition. *New Phytologist* **103**, 107-116.
- BLEDSE, C., BROWN, D., COLEMAN, M., LITTKE, W., RYGIOWICZ, P., SANGWANIT, U., ROGERS, S. & AMMIRATI, J. (1989). Physiology and metabolism of ectomycorrhizae. *Annales des Sciences Forestières* (in the press).
- COLEMAN, M. D. (1988). *Water relations of ectomycorrhizal Douglas-fir*. Ph.D. Thesis, University of Washington.
- DAVIES, W. J., METCALFE, J. C., SCHURR, U., TAYLOR, G. & ZHANG, J. (1987). Hormones as chemical signals involved in root to shoot communication of effects of changes in the soil environment. In: *Hormone Action in Plant Development - A Critical Appraisal*. (Ed. by G. V. Goad, J. R. Lenton, M. B. Jackson & R. K. Atkin), pp. 201-216. Butterworths, London.
- DIXON, R. K., GARRETT, H. E. & COX, G. S. (1988a). Cytokinin activity in *Citrus jambhiri* Lush. seedlings colonized by vesicular-arbuscular mycorrhizal fungi. *Trees* **2**, 39-44.
- DIXON, R. K., GARRETT, H. E. & COX, G. S. (1988b). Cytokinins in the root pressure exudate of *Citrus jambhiri* Lush. colonized by vesicular-arbuscular mycorrhizae. *Tree Physiology* **4**, 9-18.
- DIXON, R. K., WRIGHT, G. M., BEHRNS, G. T., TESKEY, R. O. & HINCKLEY, T. M. (1980). Water deficits and root growth of ectomycorrhizal white oak seedlings. *Canadian Journal of Forest Research* **10**, 545-548.
- FARNUM, P. (1977). *Post-planting water relations of container-grown seedlings. A mathematical model using finite elements*. Ph.D. Thesis, University of Washington.
- FISCUS, E. L. (1977). Determination of hydraulic and osmotic properties of soybean root systems. *Plant Physiology* **59**, 1013-1020.
- FISCUS, E. L. (1981). Effects of Absciscic Acid on the hydraulic conductance of and the total ion transport through *Phaseolus* root system. *Plant Physiology* **68**, 169-174.
- FISCUS, E. L. (1986). Diurnal changes in volume and solute transport coefficients of *Phaseolus* roots. *Plant Physiology* **80**, 752-759.
- FISCUS, E. L. & MARKHART, A. H. III (1979). Relationships between root system water transport properties and plant size in *Phaseolus*. *Plant Physiology* **64**, 770-773.
- FITTER, A. H. (1988). Water relations of red clover *Trifolium pratense* L. as affected by VA mycorrhizal infection and phosphorus supply before and during drought. *Journal of Experimental Botany* **39**, 595-603.
- GRAHAM, J. H. & SYVERTSEN, J. P. (1984). Influence of vesicular-arbuscular mycorrhiza on the hydraulic conductivity of roots of two citrus rootstocks. *New Phytologist* **97**, 277-284.
- GRAHAM, J. H., SYVERTSEN, J. P. & SMITH, M. L. (1987). Water relations of mycorrhizal and phosphorus-fertilized non-mycorrhizal *Citrus* under drought stress. *New Phytologist* **105**, 411-419.
- HARDIE, K. & LEYTON, L. (1981). The influence of vesicular-arbuscular mycorrhiza on growth and water relations of red clover. I. In phosphate deficient soil. *New Phytologist* **89**, 599-608.
- HARLEY, J. L. & SMITH, S. E. (1983). *Mycorrhizal Symbiosis*. Academic Press, London.
- KLEINBAUM, D. G. & KUPPER, L. L. (1978). *Applied Regression Analysis and Other Multivariable Methods*. Duxbury Press, Boston.
- KOIDE, R. (1985). The effect of VA mycorrhizal infection and phosphorus status on sunflower hydraulic and stomatal properties. *Journal of Experimental Botany* **36**, 1087-1098.
- LEVY, Y. & KRIKUN, J. (1980). Effect of vesicular-arbuscular mycorrhiza on *Citrus jambhiri* water relations. *New Phytologist* **85**, 25-31.
- LEVY, Y., SYVERTSEN, J. P. & NEMEC, S. (1983). Effects of drought stress and vesicular-arbuscular mycorrhiza on citrus transpiration and hydraulic conductivity of roots. *New Phytologist* **93**, 61-66.
- MANSFIELD, T. A. (1987). Hormones as regulators of water balance. In: *Plant Hormones and Their Role in Plant Growth and Development* (Ed. by P. J. Davies), pp. 411-430. Martinus Nijhoff, Boston.
- MARKHART, A. H. III, FISCUS, E. L., NAYLOR, A. W. & KRAMER, P. J. (1979). Effect of abscisic acid on root hydraulic conductivity. *Plant Physiology* **64**, 611-614.
- MARX, D. H. & BRYAN, W. C. (1975). Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infected with the fungal symbiont *Pisolithus tinctorius*. *Forest Science* **21**, 245-254.
- NELSEN, C. E. & SAFIR, G. R. (1982). The water relations of well-watered mycorrhizal, and non-mycorrhizal onion plants. *Journal of the American Society for Horticultural Science* **107**, 271-274.
- PARKE, J. L., LINDERMAN, R. G. & BLACK, C. H. (1983). The role of ectomycorrhizas in drought tolerance of Douglas-fir seedlings. *New Phytologist* **95**, 83-95.
- PARSONS, L. R. & KRAMER, P. J. (1974). Diurnal cycling in root resistance to water movement. *Physiologia Plantarum* **30**, 19-23.
- RADIN, J. W. & EIDENBOCK, M. P. (1984). Hydraulic conductance as a factor limiting leaf expansion of phosphorus-deficient cotton plants. *Plant Physiology* **75**, 372-377.
- SANDS, R., FISCUS, E. L. & REID, C. P. P. (1982). Hydraulic properties of pine and bean roots with varying degrees of suberization, vascular differentiation and mycorrhizal infection. *Australian Journal of Plant Physiology* **9**, 559-569.
- SANDS, R. & THEODOROU, C. (1978). Water uptake by mycorrhizal roots of radiata pine seedlings. *Australian Journal of Plant Physiology* **5**, 301-309.
- SMIT, B. & STACHOWIAK, M. (1988). The effects of hypoxia and elevated carbon dioxide levels on water flux through *Populus* roots. *Tree Physiology* **4**, 153-165.
- SMIT, B. A., NEUMAN, D. S. & STACHOWIAK, M. L. (1990). Root hypoxia reduces leaf growth: the role of factors in the transpiration stream. *Plant Physiology* (in the press).
- STEEL, R. G. D. & TORRIE, J. H. (1980). *Principles and Procedures of Statistics*. McGraw-Hill, New York.
- TORREY, J. G. (1976). Root hormones and plant growth. *Annual Review of Plant Physiology* **27**, 435-459.
- VAN DEN DRIESCHE, R. (1984). Soil fertility in forest nurseries. In: *Forest Nursery Manual*. (Ed. by M. L. Duryea & T. D. Landis), pp. 63-74. Martinus Nijhoff, Boston.